

IN THE SPECIFICATION

Please replace the paragraph bridging pages 7 and 8 with the following new paragraph:

Figure 3. Inhibition of endothelial tube formation by a mAb to DPPIV. **a**, Morphology of HUVEC tube formation in ~~Matrigel~~ Matrigel® (basement membrane matrix) assay (Control). It could be blocked by mAb E19 or E26 (DPPIV). Bar = 100 μ m. **b**, Inhibition of endothelial tube formation. All antibodies, mAb E26 (against DPPIV), C27 (β 1), E3 or C37 (control IgG), or buffer alone (Control) were applied at 5 μ g per ml prior to tube formation when cells adhered to ~~Matrigel~~ Matrigel® (basement membrane matrix). The matrix metalloprotease inhibitor CT1847 was added at 10 nM in the presence of 0.01% DMSO and 0.01% DMSO was used as vehicle control (+DMSO). Three experiments for each antibody or inhibitor were used in this plot. Tube formation was quantified by measuring the areas of tubes in each well. The values are mean \pm SD. **c**, Inhibition of preexisting endothelial tubes by antibodies to DPPIV and β 1 or the matrix metalloprotease inhibitor CT1847. Experimental conditions were identical to above except antibodies and inhibitors were applied after tubes were formed. **d**, Immunofluorescent distribution of seprase in migratory HUVEC (indicated by arrow) from a tube in ~~Matrigel~~ Matrigel® (basement membrane matrix). The HUVEC culture (phase contrast image shown in the left panel) was stained with antibodies against seprase (D8) (right panel), respectively. Bar = 10 μ m.

Please replace the paragraph bridging pages 11 and 12 with the following new paragraph:

As used herein, "anti-tumor drug" means any agent useful to combat cancer including, but not limited to, cytotoxins and agents such as antimetabolites, alkylating agents, anthracyclines, antibiotics, antimitotic agents, procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons and radioactive agents. As used herein, "a

cytotoxin or cytotoxic agent" means any agent that is detrimental to cells. Examples include Taxol[®] (paclitaxel) ~~taxol~~, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Please replace the paragraph on page 18, starting at line 9 and ending at line 14 with the following new paragraph:

Methods: Following methods were carried out as described: immunohistological staining of tissue sections (Kelly et al., 1998); seprase/DPPIV protein and proteolytic activity (Pineiro-Sanchez et al., 1997); RT-PCR (Goldstein et al., 1997); double-labeled immunofluorescence of cultured cells and β 1-integrin blotting (Mueller et al., 1999), MMP-2 activity (Nakahara et al., 1997); endothelial migration and monolayer wound assays (Pepper et al., 1996); HUVEC (Human umbilical vein endothelial cell) culture and ~~Matrigel~~ Matrigel[®] (basement membrane matrix) tube assay (Grant et al., 1992).

Please replace the paragraph on page 20, starting at line 11 and ending at line 19 with the following new paragraph:

Example 5: Inhibitory effects of anti-DPPIV mAbs on blood vessel tube formation. To examine the effects of these same antibodies on endothelial tube formation by ~~Matrigel~~ Matrigel[®] (basement membrane matrix) (Grant et al., 1992), mAbs or the matrix metalloprotease inhibitor CT1847 (Zucker et al., 1998) were added prior to or after tube formation (Fig. 3a). The inhibitory anti-DPPIV and β 1 mAbs and CT1847 blocked tube formation in ~~Matrigel~~ Matrigel[®] (basement membrane matrix) (Fig. 3b); however, only anti- β 1 mAb and CT1847 but not the anti-

DPPIV mAb perturbed preexisting tubes (Fig. 3c). None of the other mAbs to DPPIV and seprase affected preexisting endothelial tubes. Specific expression of seprase and DPPIV in the endothelial cell migrated from a forming tube (Fig. 3d) also supports the observation that the anti-DPPIV mAb appears to act selectively on new tube formation.

Please replace the paragraph on page 21, starting at line 4 and ending at line 20 with the following new paragraph:

Example 7: The active domain of the Seprase-DPPIV complex. Recent cloning studies (Goldstein et al., 1997) show that seprase carboxyl terminus contains a putative catalytic region (~200 amino acids), which is homologous (68% identity) to that of the non-classical serine peptidase DPPIV. The conserved serine protease motif G-X-S-X-G (SEQ. ID. NO: 1) is present as G-W-S-Y-G (SEQ. ID. NO: 2). Like DPPIV, seprase have 12 Cys with 6 residues being conserved in the Cys rich region and 3 in the catalytic region. Seprase has a peculiar protease inhibitor profile: it is inhibited by the protease inhibitors, including PMSF and NEM (Aoyama and Chen, 1990). Its gelatinase activity was completely blocked by the serine-protease inhibitors, DFP, PMSF, AEBSF, and APSF. Dimeric seprase could be affinity-labeled by [³H]-DFP but the proteolytically inactive 97-kDa subunit could not (Pineiro-Sanchez et al., 1997). The inhibitor and substrate specificity of the seprase-DPPIV complex isolated from human breast carcinoma cells was analyzed by [³H]-DFP labeling. The method is extremely sensitive in detecting serine proteases and esterases (10^{-13} M) and is based upon the stoichiometrical, covalent binding of [³H]-DFP into the proteases that are reduced in the presence of their substrates and inhibitors. Both dimeric seprase and DPPIV may be labeled with [³H]-DFP and their molecular identity may be visualized on SDS gels (Pineiro-Sanchez et al., 1997). By incubating the seprase-DPPIV complex with [³H]-DFP in the presence of their peptide-substrates or inhibitors, the protease inhibition is quantified.

Please replace the paragraph bridging pages 24 and 25 with the following new paragraph:

Example 12: General Procedure for Conjugating Small Molecular Drugs to an Antibody.

Antibody-small molecule conjugates are prepared by linking the DOX derivative maleimidocaproyl doxorubicin hydrazone or the maleimidocaproylhydrazone of ~~Adriamycin~~ Adriamycin® (doxorubicin) to E19, E26 or control immunoglobulin following the procedure of Hellstrom, US Patent No., 5,980,896. Antibody is diluted with 0.0095 M PBS to a protein concentration of 10.49 mg/mL. This solution (500 mL) is heated to 37°C., under a nitrogen atmosphere, in a water bath. Dithiothreitol (26.2 mL, 10 mM) in PBS is added and the solution is stirred for 3 hrs at 37°C. The solution is divided equally between two Amicon Model 8400 stirred ultrafiltration cells each fitted with a YM 30 ultrafilter (MW cutoff 30,000, 76 mm diam.) and connected via a Model CDS10 concentration/dialysis selector to a Model RC800 mini-reservoir (Amicon, Division of W. R. Grace and Co., Beverly, MA). Each reservoir contains 800 mL of 0.0095 M PBS-0.1 M L-histidine. The protein solutions are dialyzed until the concentration of free thiol in the filtrate was 63 .mu.M. The molar ratio of --SH/protein in the retentate is determined to be 8.16. The retentate is transferred from the cells to a sterile container under nitrogen and a solution of maleimidocaproyl hydrazone of ~~adriamycin~~ Adriamycin® (doxorubicin) (42.6 mL, 5 mg/mL in water) is added with stirring. The conjugate is incubated at 4°C. for 48 hrs after which it is filtered through a 0.22.mu. cellulose acetate membrane. A 2.5 cm.x.50 cm Bio-Rad Econocolumn is packed with a slurry of 100 g of BioBeads.TM.SM-2 (Bio-Rad Laboratories, Richmond Calif. 94804) in 0.0095 M-0.1 M L-histidine buffer. The beads are prepared by washing in methanol, followed by water then several volumes of buffer. The filtered conjugate is percolated through this column at 2 mL/min. After chromatography the conjugate is filtered through a 0.22.mu. cellulose acetate membrane, frozen in liquid nitrogen and stored at -80°C. The conjugate obtained has a molar ratio of 6.77 ~~Adriamycin~~ Adriamycin® (doxorubicin) to protein and is obtained in 80-95% yield.

Please replace the paragraph on page 26, starting at line 15 and ending at line 24 with the following new paragraph:

The experimental human angiogenesis model is prepared essentially as described in Yan, et al., J. Clin. Invest., 91:986-996 (1993). Briefly, a 2 cm² square area of skin is surgically removed from a SCID mouse (6-8 weeks of age) and replaced with a human foreskin. The mouse is anesthetized and the hair removed from a 5 cm² area on each side of the lateral abdominal region by shaving. Two circular graft beds of 2 cm² are prepared by removing the full thickness of skin down to the fascia. Full thickness human skin grafts of the same size derived from human neonatal foreskin are placed onto the wound beds and held in place with 5-0 monofilament suture (~~Dermalon~~ Dermalon[®], Davis and Geck Inc., Danbury, CT). The graft is covered with a ~~Band-Aid~~ Band-Aid[®] (bandage), which is sutured to the skin. Micropore surgical tape (3M, St. Paul, MN) is also applied to cover the wound. Mice are housed in individual cages.

Please replace the paragraph bridging pages 29 and 30 with the following new paragraph:

Using the ribozyme approach, DPPIV expression was abrogated in invasive cells to examine the influence of individual membrane proteases on tumor cell proliferation and invasiveness. A hammerhead ribozyme usually consists of a catalytic center made up of a highly conserved sequence of 22 nucleotides and two flanking regions that base-pair the ribozyme with targeted RNA sequences flanking the trinucleotide GUX, where X can be A, C or U. A ribozyme normally cleaves on the 3' side of the trinucleotide in the targeted RNA (Haseloff and Gerlach, 1988). To generate ribozyme constructs targeted at DPPIV, the cDNA sequence was scanned and potential cleavage sites identified. A pair of sense and anti-sense oligonucleotides with complimentary 3' ends, covering the sequences of the catalytic center and flanking regions of the cleavage site have been synthesized. As an example, the nucleotide sequences of sense and antisense oligonucleotides used to construct the ribozyme #8 (rbz#8) targeting a site in the seprase sequence are shown as follows:

5'-AGGCACTGAACTGATGAGTCCGTGAGG-3' (SEQ. ID. NO: 3) Sense
oligonucleotide

5'-TGAAGAGGAAGTTTCGTCCTCACGG-3' (SEQ. ID. NO: 4) Antisense
oligonucleotide

Please replace the paragraph on page 33, starting at line 3 and ending at line 20 with the following new paragraph:

To investigate the expression of the seprase-DPPIV complex during cancer invasion, angiogenesis and tissue repair, human wound granulation tissue or adjacent normal skin and infiltrating ductal carcinomas or their corresponding adjacent normal tissues were examined. Human gingival biopsies were derived from the University of Turku, Finland. Full thickness wounds of oral mucosa were made from two healthy volunteers and biopsies were collected after 3, 7, 14 and 28 days of wounding. Immediately after biopsy, fresh tissue blocks were mounted in Histoprep® (Fisher Scientific, New Jersey) and snap frozen in liquid nitrogen. Frozen sections (6 µm) were cut, fixed with acetone at -20°C for 5 min, and stored at -70°C. For routine histology, the sections were stained with hematoxylin and eosin. For immunohistochemical staining, sections were washed with PBS containing 0.1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) and incubated with rhodamine-conjugated mAb D28 against seprase or mAb E19 against DPPIV in PBS/BSA in humid chamber at 4°C for 16 h. The sections were then washed with PBS/BSA and water, briefly air-dried, and mounted using cyanoacrylate glue (~~Krazy Glue~~ Krazy Glue®, Borden Company LTD). The staining was examined using a Zeiss Axioskop 20 light, fluorescence and confocal microscopy, and photographed using MC 80 Zeiss microscope camera. Control staining was performed with rhodamine-conjugated secondary antibody and showed no specific stain. Immunohistochemical staining of invasive human breast carcinoma was performed as described (Kelly et al., 1998).

Please replace the paragraph on page 34, starting at line 3 and ending at line 24 with the following new paragraph:

Immunohistochemistry of human breast invasive carcinoma and adjacent normal tissue:
The immunohistochemistry procedure involves fixation of invasive breast cancer and adjacent tissues with 4% paraformaldehyde in PBS for 2-4 h at 4°C, followed by paraffin embedding. Paraffin-embedded tissue blocks were sectioned in 4- μ m-thick pieces using a microtome. The samples were adhered to glass slides (Matsunami, Tokyo, Japan) and dried at 42°C overnight. The slides were cooled and de-paraffinized through three changes of ~~Americlear~~ Americlear® (histology cleaning solution) (Baxter, Deerfield, IL). Then the slides were rinsed in 100% ethanol twice and 95% ethanol twice and re-hydrated with distilled water. Antigens were retrieved by treating slides in a container covered with 10mM sodium citrate buffer, pH6.0, and heated at 120°C for 5 min by autoclave. After heat treatment, the buffer was allowed to cool down to normal temperature, and replaced with PBS. The slides were then treated with 10% normal blocking serum in PBS for 15-30 min. Blocking serum should be derived from the species in which the secondary antibody was made: normal horse serum was used for primary mouse monoclonals, and normal rabbit serum for primary rat monoclonals. Anti-seprase mAbs D8 or D28 or anti-DPPIV mAbs E19 or E26 in serum-free supernatant form were added at a dilution of 1:10 to 1:25 to each tissue section and incubated at 4°C overnight in a humidity chamber. Mouse mAb against human endothelial cells (CD34, Cosmo Bio Co. Ltd., Tokyo, Japan) were added at a dilution of 1:50; and a mixture of three mouse mAbs, including C11 (1:400), ESA (1:50) and EMA (1:60) were used for marking epithelial cells. Bound primary antibody was then detected by streptavidin-biotin-peroxidase technique (DAKO) according to the manufacturer's instructions using diaminobenzidine (3,3'-diaminobenzidine tetrahydrochloride, Sigma, St. Louis, MO) as a chromogen and counter-staining was performed with hematoxylin.

Please replace the paragraph on page 37, starting at line 7 and ending at line 31 with the following new paragraph:

Example 18: *In vivo* regression of human tumor growth and angiogenesis with DPPIV antagonists as measured by a chimeric mouse-human assay. The effects of monoclonal antibodies on human tumor growth and angiogenesis were assessed by co-inoculating human breast carcinoma cell line MDA-MB-436 (seprase+/DPPIV+) or human malignant melanoma cell line LOX (seprase+/DPPIV-) or ovarian cancer cells (seprase+/DPPIV+) with endothelial cells (CD31+) isolated from ovarian cancer ascites into the skin of SCID or nude mice. Severe combined immunodeficiency (SCID) mice, C.B-17-scid strain, were used in this assay. SCID lack both T and B cells due to a defect in V(D)J recombination, and SCID mice do not mount an antibody response to challenge by immunogenic material. Therefore, they easily accept foreign tissue transplants, including human cells. The SCID-human model was prepared essentially as described in (Yan et al., 1993). Briefly, seprase+/DPPIV+ and seprase+/DPPIV- cells tagged with lacZ were mixed with CD31+ human endothelial cells in ~~Matrigel~~ Matrigel® (basement membrane matrix) (2×10^6 cells each cell type), in the presence of monoclonal antibodies or inhibitors, were subcutaneously injected into 6-8 week-old SCID mice. Mice were housed in individual cages. The mice were then observed for 2 to 4 weeks to allow growth of measurable human tumors. Following the growth of measurable tumors, SCID mice were injected intravenously into the tail vein with 250 μ g of either the mAb E19, E29 or E3 (anti-DPPIV) twice a week for 2 to 3 weeks. After this time, the tumors were resected from the skin and trimmed free of surrounding tissue. Three to four mice were used for each treatment and the result of suppression of tumor growth (as determined by size) with DPPIV antagonist antibodies are shown in Table I. The color of the tumor and immunohistochemistry results were used to indicate the degree of tumor angiogenesis. In this model, human tumor growth and neovascularization were occurring in the mouse tissue. The cell origin of the neovascularization within the human tumor was demonstrated by immunohistochemical staining of the neovasculature with human-specific endothelial cell markers, including anti-human factor VIII and CD31 antibodies.